Author's response to reviews

Title: Abnormal energy regulation in early life: Childhood gene expression may predict subsequent chronic mountain sickness

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Author's response to reviews: see over
Lima, October 9, 2008

Rikki Graham, PhD
Senior Assistant Editor
BMC-series journals

REF: MS: 1503895877211738 - Abnormal energy regulation in early life: Childhood gene expression predicts subsequent chronic mountain sickness

Dear Dr. Graham,

Many thanks for your email dated October 08, 2008 regarding our manuscript.

Please find below our point-by-point replies to both reviewers´ comments. We have also made the changes to the manuscript, according to reviewers´ suggestions.

Yours sincerely,

Luis Huicho
Reviewer 1

**Title:** Abnormal energy regulation in early life: Childhood gene expression predicts subsequent chronic mountain sickness
**Version:** 3  **Date:** 18 August 2008  
**Reviewer:** Hilde Spielvogel

**Reviewer’s report:**
1. The hypothesis posed by the authors is well defined.
2. The methods are appropriate and clearly described in detail.
3. The data are sound and well presented.
4. The manuscript follows the relevant standards for reporting and data deposition.
5. The discussion and conclusions are well balanced and adequately supported by the data.
6. The authors have clearly stated limitations of their work by mentioning methodological constraints referring to strict laboratory procedures in the field, and time limitations at altitude for sea level researchers.
7. The authors have cited 25 related articles. The present paper is based on their own previous work.
8. I have a problem with the title. The authors write in the conclusion that they designed the study to measure gene expression that may predict the later occurrence of CMS. I do agree with this statement and therefore I think this should also be conveyed in the title which should say Childhood gene expression may predict subsequent chronic mountain sickness. The Abstract is accurate.

**Answer:** We changed the title as Reviewer asked.

9. The article is well written.
Under Minor Essential Revisions I would like the authors to change the following:
1. In the Abstract (line 2) they write “CMS a maladaptation syndrome in native highlanders”. CMS, however, can also occur in sea level natives during prolonged residence at high altitude. In fact, Carlos Monge described the disease for the first time in an engineer from Lima who had worked during one year in Cerro de Pasco when he increased the number of erythrocytes above the level normally found in healthy high altitude natives.

**Answer:** Thanks to Reviewer for pointing this out. We modified the sentence in the Abstract and it states now: “… a clinical condition that occurs to native highlanders or to sea level natives with prolonged residence at high altitude”.

2. References are cited throughout the text by numbers in parenthesis with the exception of Introduction on page 3 (line 14) and on page 15 (line 4, where they are in superscript (?)). This should be corrected.

**Answer:** All references are in square brackets now, according to the journal requirements.

3. On page 10 it should say Krebs cycle.

**Answer:** Corrected now.
Reviewer 2

Reviewer’s report
Title: Abnormal energy regulation in early life: Childhood gene expression predicts subsequent chronic mountain sickness
Version: 3 Date: 27 September 2008
Reviewer: Gustavo R Zubieta-Calleja

Reviewer’s report:

Major Compulsory Revisions

The use of the term “maladaptation syndrome” in reference to Chronic Mountain Sickness in the second sentence of the Abstract is a mistake in our opinion. A serious flaw as this has been part of a subject of discussion in the International Consensus Group in Xining (2), where we had a dissenting point of view. The term “Loss of Adaptation” as originally stated by Carlos Monge in his first descriptions of CMS over 80 years ago was, after long and difficult discussions, dropped from the statement.

“The organic systems of human beings and all other species tend to adapt to any environmental change and circumstance within an optimal period of time, and never tend towards regression which would inevitably lead to death” (1)

We stated our concepts in regards to CMS within the aforementioned group (comments are available, on-line at http://www.geocities.com/CapeCanaveral/6280/cmsdisc.html).

Consequently, there is too much speculation about CMS, yet not clearly defined as a unique individual entity but rather due to multiple diseases.

Polyerythrocythemia (increase of red blood cells above normal values, previously known as excessive erythrocytosis, increased polycythemia, erythremia) is a sign that arises from diverse pulmonary, cardiac, renal, hematological, neurological alterations that at high altitude phenotypically expose the disease. This fundamentally due to the steep portion of the oxygen dissociation curve.

Answer: We removed the term “maladaptation” from the Abstract, and the sentence states now: “...a clinical condition that occurs to native highlanders or to sea level natives with prolonged residence at high altitude” (Abstract, lines 2-3).

It is well known that genetics pre-dispose to specific disease. For example Cancer. However one should consider that there are trigger factors that “activate” the disease. AS it is also evident that not all those that have a genetic predisposition to CA will indeed have it. Lung CA is a good example, because heavy smoking is the trigger.

Answer: We agree with this general and widely accepted viewpoint. For the more specific issue on genes and hypoxia, see our more detailed reply below.

Noteworthy is the fact that no link has been found between PHD3 (marker D14S1049) along with other candidate genes known to be involved with hypoxia sensing and erythropoiesis, erythropoietin, erythropoietin-receptor, HIF1a, Von Hippel-Lindau, propyl hydroxilase domain containing 1,2,3 and phosphatase and tensin homolog deleted on chromosome ten in CMS patients. (3)
**Answer:** We were aware of the study performed by Mejia and col and cited by Reviewer. They in fact used a different approach to ours, focusing on functional candidate genes that could account for the increased erythropoietic response observed in CMS patients (EPO, EPOR, HIF1A, PHD1, PHD2, PHD3, VHL, and PTEN). We focused instead on oxygen-responsive genes and on those involved in glycolytic and mitochondrial metabolic pathways.

Since CMS is an undefined disease, according to our experience without any genetical factor, CMS can be present and is more severe, in many types of respiratory and ventilatory disease. We are familiar with most of the CMS bibliography and we personally know the majority of the authors and cannot fail to notice that mistakes are often made, selecting patients with CMS that clearly had pulmonary disease (4). This systematic mistake has lead the International Consensus Group to make a score for the classification of CMS. We strongly expressed our disagreement, CMS greatly differs from AMS which can indeed have a scoring system such as the Lake Louise, since it is acute and with limited symptomatology, being hypoxia the only trigger. At the conclusion of our international meetings (2) in several parts of the world and through the internet, we were invited by Jack Reeves to publish another paper expressing our points of view: “Chronic Mountain Sickness: The reaction of physical disorders to chronic hypoxia”. (1).

Considering CMS has multiple ethiopathogenesis, hardly can one search for a specific gene. CMS when intensively examined present cardio-pulmonary pathologies that are often overlooked. To find a specific gene in children of one male parent with CMS, one could well be looking at a gene that gives rise to intrapulmonary shunts, or chronic bronchitis, or any other disease that in the hypoxic environment of high altitude would give rise to polycythaemia (commonly known as CMS).

Hypoxia as a trigger of CMS, not only depends on hypoxia but likewise on the magnitude of the respiratory and ventilatory lesions. For example, a small pulmonary lesion will not give rise to CMS at 3000 m but will become evident above that altitude.

**Answer:** The reviewer extensively offers his views on a controversy about the definition, nature and pathophysiology of CMS, and the ways to diagnose it and to determine its severity (such as the Consensus score referred to). We agree with him that all studies on CMS should be performed carefully, so as to avoid selection bias leading to inclusion of patients with other clinical conditions that may also modify the erythropoietic response. As this is a pediatric paper, we did not include patients with CMS, but we stated clearly in our recent paper reporting on male parents of our children that we excluded subjects with any significant acute or chronic clinical condition (reference 11 of our paper).

We also concur with the reviewer that genetic predisposition to CMS can hardly be explained by one single genetic alteration. Modifying factors (life style, environmental and indoor pollution, and chronic respiratory diseases) and programming (the long-term indirect effects that poverty, malnutrition and other environmental agents may exert on the responses to hypoxia in the fetal and early postnatal vulnerable periods of life) can change substantially the genetic expression of both acute and long-term responses. We discuss in detail this
important issue in a recent paper we published (reference 12 in the current paper). In fact, and as an acknowledgement of the importance of non-genetic factors, what we state in the Discussion of our paper (page 14, paragraph 3) is that gene expression levels, which we measured in our study (not genetic mutations), are the result of many factors that act on the DNA sequence modifying thus its phenotypic expression. Such factors include epigenetic influences not involving DNA mutations, and other environmental factors acting during embryonic, fetal, or early postnatal periods that may also modify gene expression.

The children in this study are defined as subjects that will not adapt to high altitude, and this seems incongruent with the reality that they will indeed live on, to become adults at high altitude, just as their parents. **Answer:** The reviewer rightly argues that it would be wrong to state without reserve that our study children will not adapt to high altitude when they reach adulthood. In fact, in both Discussion and Conclusion sections, we emphasize (in conditional) that our results **may** predict the later occurrence of CMS, regardless of how such biomarkers may have been induced, by epigenetic influences, evolutionary pressures or failure to respond to hypoxic stress.